New Antiprogestins with Partial Agonist Activity: Potential Selective Progesterone Receptor Modulators (SPRMs) and Probes for Receptor- and Coregulator-Induced Changes in Progesterone Receptor Induction Properties

Georgia Giannoukos*, Daniele Szapary, Catharine L. Smith, James E. W. Meeker†, and S. Stoney Simons, Jr.

Steroid Hormones Section (G.G., D.S., J.E.W.M., S.S.S.)
Laboratory of Molecular and Cellular Biology
National Institute of Diabetes, Digestive and Kidney Diseases and the
Laboratory of Receptor Biology and Gene Expression (C.L.S.)
National Cancer Institute/Division of Basic Sciences
National Institutes of Health
Bethesda, Maryland 20892-0805

A pharmacologically relevant property of steroid hormone-regulated gene induction is the partial agonist activity of antisteroid complexes. We now report that dexamethasone-mesylate (Dex-Mes) and dexamethasone-oxetanone (Dex-Ox), each a derivative of the glucocorticoid-selective steroid dexamethasone (Dex), are two new antiprogestins with significant amounts of agonist activity with both the A and B isoforms of progesterone receptor (PR), for different progesterone-responsive elements, and in several cell lines. These compounds continue to display activity under conditions where another partial antiprogestin (RTI-020) is inactive. These new antiprogestins were used to determine whether the partial agonist activity of PR complexes can be modified by changing concentrations of receptor or coregulator, as we have recently demonstrated for glucocorticoid receptors (GRs). Because GR and coregulator concentrations simultaneously altered the position of the physiologically relevant dose-response curve, and associated EC_{50}, of GR-agonist complexes, we also examined this phenomenon with PR. We find that elevated PR or transcriptional intermediary factor 2 (TIF2) concentrations increase the partial agonist activity of Dex-Mes and Dex-Ox, and the EC_{50} of agonists, independently of changes in total gene transactivation. Furthermore, the corepressors SMRT (silencing mediator for retinoid and thyroid receptors) and NCoR (nuclear receptor corepressor) each suppresses gene induction but NCoR acts opposite to SMRT and, like the coactivator TIF2, reduces the EC_{50} and increases the partial agonist activity of antiprogestins. These comparable responses of GR and PR suggest that variations in receptor and coregulator concentrations may be a general mechanism for altering the induction properties of other steroid receptors. Finally, the magnitude of coregulator effects on PR induction properties are often not identical for agonists and the new antagonists, suggesting subtle mechanistic differences. These properties of Dex-Mes and Dex-Ox, plus the sensitivity of their activity to cellular differences in PR and coregulator concentrations, make these steroids potential new SPRMs (selective progesterone receptor modulators) that should prove useful as probes of PR induction properties. (Molecular Endocrinology 15: 255–270, 2001)

0888-8809/01/$3.00/0
Molecular Endocrinology 15(2): 255–270
Copyright © 2001 by The Endocrine Society
Printed in U.S.A.

INTRODUCTION

A common use of endocrine therapies in the clinical setting is to block the action of an endogenous hormone in both normal and malignant tissues. The inhibition of progesterone action in women is used to prevent conception at a variety of stages (1, 2). The growth of many breast cancer tumors is retarded by suppressing the action of endogenous estrogens (3, 4). Pure antisteroids, which display no agonist activity, represent one of the most straightforward methods of blocking the actions of steroid hormones. The antisteroid undergoes most of the same steps as agonist steroids, including steroid binding to the cognate receptor, activation, nuclear translocation, and DNA binding (5, 6). It is predominantly in the association with coregulators and the transcriptional machinery that the antisteroid complexes appear to differ and prevent the changes in rates of gene transcription seen with agonist complexes (7–9). Unfortunately, the side effects of antisteroid therapies can be quite severe. A pure antagonist will block all of the actions of a particular receptor in addition to the one that is targeted. Furthermore, some antisteroids, like RU 486 (10), cross-react with other receptors to block the actions of multiple classes of steroids (11), thereby magnifying the number of undesired actions.

An exciting new approach to endocrine therapies has emerged with the concept of SRMs, or selective receptor modulators. These are compounds that are antagonists for some genes/tissues but agonists for others. Thus, they are not pure antagonists. This ability to display partial agonist activity with selected reporter genes can be clinically very beneficial. For example, the agonist activity of raloxifene and tamoxifen in bone coupled with their antagonist activity in breast (Ref. 12 and references therein) suggests that their use in the treatment of breast cancer might not be accompanied by the osteoporosis seen with other antiestrogens (13). While it is not yet possible to predict the genes for which a particular SRM will display antagonist *vs.* agonist activity, an absolute requirement is that the steroid possesses partial agonist activity for at least one gene.

Surprisingly, there are very few antiprogestins with partial agonist activity for any genes and thus are candidate-selective progesterone receptor modulators, or SPRMs. RU 486 is the most commonly used antiprogestin and displays partial agonist activity only under selected conditions (14, 15). More recently, a closely related derivative of RU 486, called RTI 3021-020 (RTI-020), was found to be a partial agonist in T47D cells but was not active in CV-1 cells (16). Therefore, it would be extremely helpful for theoretical and clinical studies to identify new antiprogestins with partial agonist activity.

Another approach for modifying the activities of antisteroids involves the coregulators that appear to be recruited by DNA-bound receptors to help modify the rates of target gene transcription. Ligand-free nuclear receptors, and some steroid receptors bound by antagonists (9, 15, 17), are usually associated with the corepressors SMRT (signal mediator and repressor of transcription) or NCoR (nuclear receptor corepressor) (18, 19). Agonist binding is thought to cause the release of corepressors and allow the association of coactivators (20–24). While corepressors are known to influence the partial agonist activity of antisteroid complexes (8, 9, 15, 17, 25, 26), an effect of coactivators on the activity of receptor-antisteroid complexes has only lately been seen with GRs (26, 27). Interestingly, different concentrations in coregulators also alter the concentration of the glucocorticoid dexamethasone (Dex) required for half-maximal induction, or EC_{50} (26, 27). Variations in the EC_{50} are highly significant for cellular functions because physiological concentrations of steroid are subsaturating and much closer to the EC_{50} than to the saturating concentrations of steroid required for maximal induction by receptors. Therefore, even small differences in EC_{50}, which are associated with shifts in the position of the dose-response curve, can have significant consequences for the amount of gene induction seen with endogenous levels of steroids.

Both the EC_{50} for agonist complexes of glucocorticoid receptors (GRs) and the partial agonist activity of GR antagonist complexes have recently been shown to be additionally influenced by changes in GR concentration (26–28). These effects were independent of cell, enhancer, promoter, and reporter gene. In all cases, the changes in EC_{50} and partial agonist activity of antiglucocorticoids occurred in parallel. Therefore, at least for GRs, changes in receptor and coregulator levels can affect the induction properties of both agonist and antagonist complexes. These observations, coupled with the documented tissue variations of coregulator (25, 29–33) and GR concentrations, suggest an attractive model: that differential control of gene expression during development, differentiation, and homeostasis by GR may be achieved, at least in part, by intra- and intercellular fluctuations in GR and coregulator concentrations (26).

In this study, we report that two antiglucocorticoids, dexamethasone-21-meslyate (Dex-Mes) (34) and dexamethasone-oxetanone (Dex-Ox) (35), are new antiprogestins possessing partial agonist activity. This agonist activity is observed under conditions where other antiprogestins are inactive, thus making Dex-Mes and Dex-Ox new potential SPRMs. Using these compounds along with the progesterone receptor (PR) agonist R5020, we found that the partial agonist activity of antagonist complexes, and the EC_{50} for agonist complexes, could be modulated by different concentrations of receptor, coactivator, and corepressor just like they are for GRs (26–28). These novel methods of modifying PR activity appear to offer new tools for studies of PR action and for endocrine treatment of clinical disorders.

RESULTS

Derivatives of the Glucocorticoid Dexamethasone Are New Ligands for PR with Partial Agonist Activity

The synthetic glucocorticoid dexamethasone (Dex, Fig. 1) has very low affinity for, and no biological activity with, PR (36, 37) (data not shown). It was, therefore, very unexpected when two derivatives of Dex,

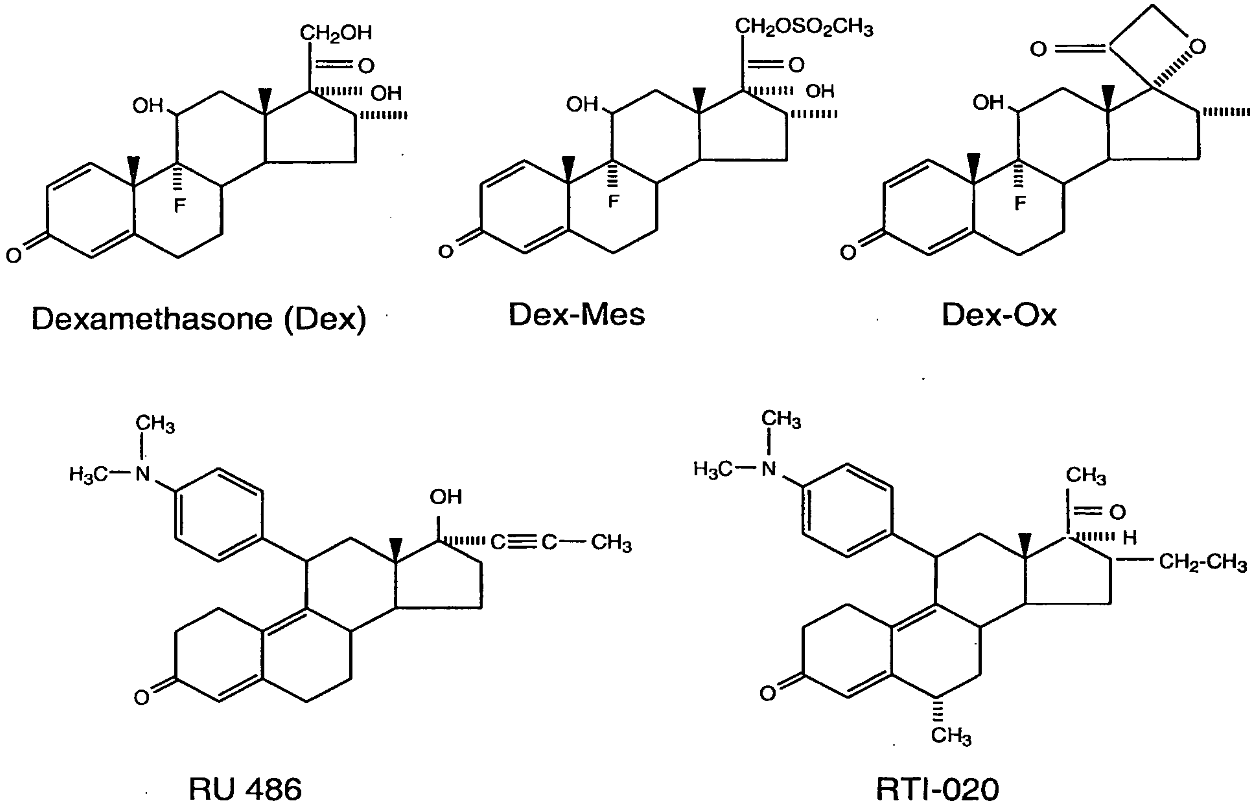

Fig. 1. Structures of Steroids Used in This Study

Dex-Mes and Dex-Ox (Fig. 1), exhibited significant quantities of agonist activity with transfected PR in CV-1 cells (Fig. 2A). Dex-Mes consistently displayed appreciable levels of partial agonist activity with both human PR isoforms (PR-A and PR-B) with two different reporter constructs (GREtkCAT and MMTVLUC). RTI-020, a derivative of RU 486 (Fig. 1), has been reported to possess partial agonist activity with PR under some conditions but not in CV-1 cells (16). Our experiments confirmed this while showing that Dex-Mes and Dex-Ox manifest significant amounts of activity under the same conditions (see PR-B with GREtkCAT in Fig. 2A).

T47D human breast cancer cells contain both PR isoforms in addition to GRs (38). When these cells were transiently transfected with the GREtkLUC reporter, sizable amounts of activity were afforded by the endogenous receptors for all of the added steroids except RU 486 (Fig. 2B). Under these conditions, Dex-Ox was much less effective than R5020, consistent with Dex-Ox being a partial agonist for PR. Dex-Ox is also a partial agonist for GR (35, 39). However, very little of the Dex-Ox agonist activity in T47D cells can be attributed to the endogenous GR as the total activity with Dex-Ox is much greater than that seen for the full glucocorticoid Dex (Fig. 2B). Dex-Mes is also a partial GR agonist (26, 28, 34, 35, 39). As the total Dex-Mes activity is less than that for Dex, we can not immediately determine which receptor(s) is responsible for the activity of Dex-Mes in T47D cells.

The above fold inductions in CV-1 cells by transiently transfected PR-A or PR-B with saturating concentrations of R5020 were low except for PR-A with the mouse mammary tumor virus (MMTV) reporter (Fig. 2A). To further investigate the properties of Dex-Mes and Dex-Ox under conditions providing more robust responses, we looked for an alternative cell line. We decided to concentrate on the larger PR isoform, PR-B, because it has been the focus of many previous studies on PR action (14, 16, 40). 1470.2 mouse mammary adenocarcinoma cells contain very little endogenous PR (38) but were found to display steady increases in total transactivation and fold induction over a range of transiently transfected human PR-B cDNA (Fig. 3A). Therefore, under these conditions, PR is limiting for transactivation. 1470.2 cells do contain endogenous GR, which binds Dex with the normal affinity of 2–3 nM (data not shown) (41, 42). These GRs are inducible by added Dex but not by any of the other steroids examined (Fig. 3B). Therefore, any partial agonist activity displayed by Dex-Mes, Dex-Ox, or RTI-020 in 1470.2 cells that are transiently transfected with PR-B would be due to PR complexes.

When 1470.2 cells are transfected with low amounts of PR-B (3 ng) and GREtkLUC reporter, significant inductions are observed for Dex-Mes and Dex-Ox, but

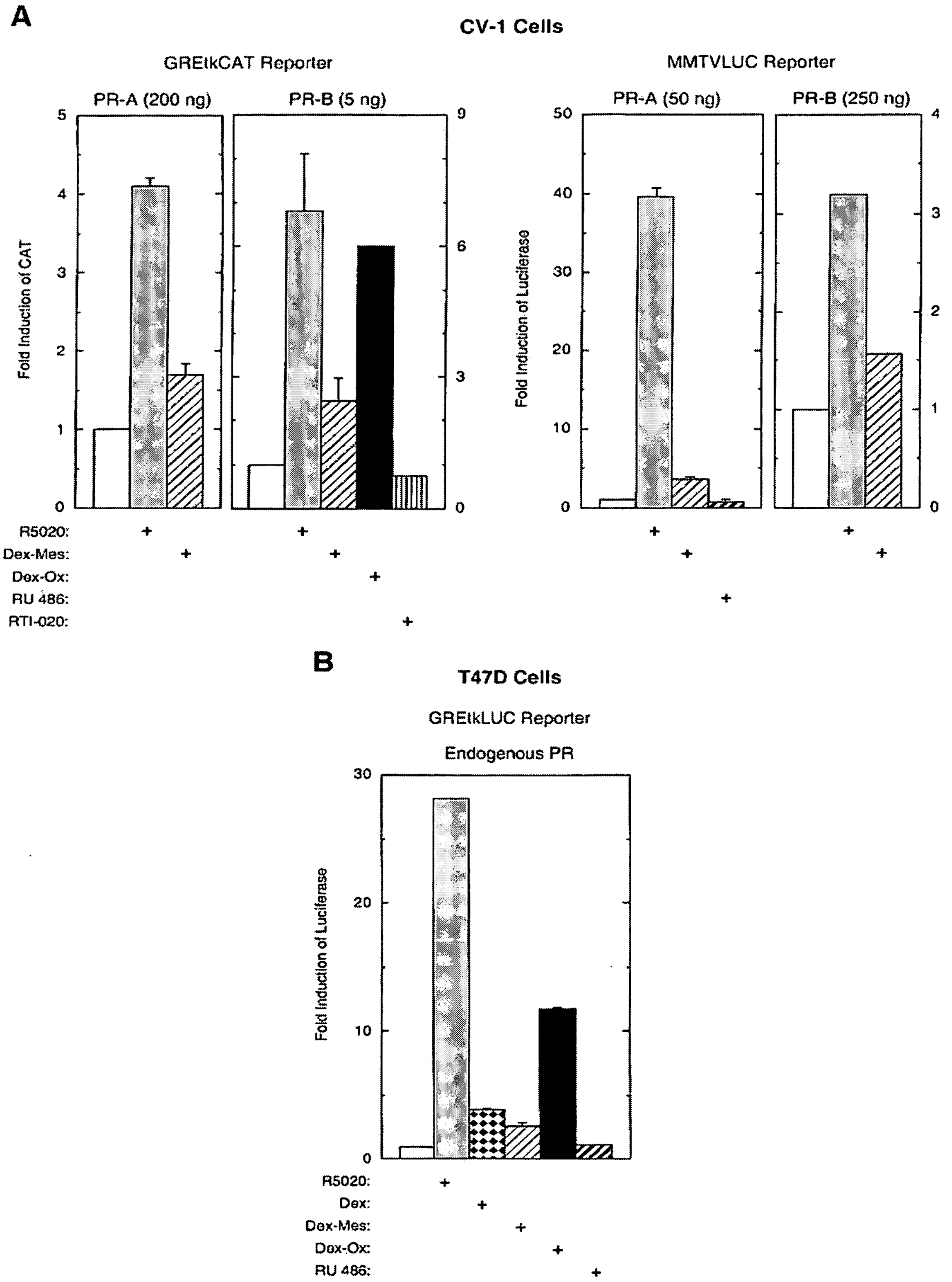

Fig. 2. Activity of Dex Derivatives with Transfected and Endogenous PR

A, Transiently transfected PR-A or PR-B with GREtkCAT or MMTVLUC reporter in CV-1 cells. Triplicate cultures were transiently transfected with GREtkCAT, or MMTVLUC, plus the indicated amounts of hPR cDNA plasmid, induced with either 30 nM R5020 or 1 μM of the indicated steroids, and assayed for luciferase activity as described in *Materials and Methods*. The total both are less than that for the full progestin R5020 (Fig. 3C). As was suggested by the data in CV-1 cells (Fig. 2A), the partial agonist activity of Dex-Ox is greater than that of Dex-Mes, and both are greater than that of RTI-020, which is almost inactive. This is shown more clearly when the data of Fig. 3C are expressed as percent of maximal activity by saturating concentrations of R5020 to display directly the percent partial agonist activity of Dex-Mes, Dex-Ox, and RTI-020 (Fig. 3D). Under these conditions in 1470.2 cells, Dex-Mes and Dex-Ox have 20% and 45% partial agonist activity, respectively, compared with less than 5% activity for RTI-020. Collectively, these data indicate that Dex-Mes and Dex-Ox are new partial agonists for PR in a manner that is independent of receptor form (PR-A or -B), of enhancer, promoter, and gene (GREtkCAT, GREtkLUC, and MMTVLuc), and of cell type (1470.2, T47D, and CV-1 cells).

Dex-Mes and Dex-Ox Are Antiprogestins That Interact with PR

The fact that Dex-Mes and Dex-Ox usually elicit less total activity than R5020 under the variety of above conditions suggested that these Dex derivatives are antiprogestins with partial agonist activity. To determine whether these effects resulted from steroid binding to PR, we examined the ability of each steroid to bind to PR and to competitively inhibit the whole cell activity of PR-agonist complexes. In a cell-free competition assay, the relative affinity for PR is found to be R5020 $\gg$ Dex-Ox $>$ Dex-Mes (Fig. 4A). From these data, one can determine that the apparent affinity of Dex-Ox for PR is at least 10-fold higher than Dex-Mes and about 100-fold lower than R5020.

Despite these low binding affinities of Dex-Mes and Dex-Ox for PR, both compounds were found to be good inhibitors of R5020 activity in intact cells. In view of the lower affinity of Dex-Mes *vs.* Dex-Ox for PR in Fig. 4A, we used 1 and 10 μM Dex-Mes, but only 1 μM Dex-Ox, to inhibit the action of 300 pM R5020 with 3 ng of transfected PR. With high enough concentrations, each Dex derivative reduces the activity of 300 pM R5020 to that of the competing steroid alone, with Dex-Ox being at least 10-fold more potent than Dex-Mes (Fig. 4B). Therefore, Dex-Mes and Dex-Ox are antiprogestins with potencies that appear to parallel their affinities for PR. This correspondence suggests that it is not a metabolite of Dex-Mes, or Dex-Ox, that is the active antiprogestin steroid. The ability of 0.3 nM R5020 to partially overcome the inactivity of 1 μM, but not 10 μM, Dex-Mes also argues that Dex-Mes is exerting its antiprogestin activity by binding directly, albeit weakly, to PR as opposed to acting via a non-PR-mediated pathway.

Effect of Changing PR Concentration on R5020 Dose-Response Curve

We previously reported that changing concentrations of GRs modulate the partial agonist activity of several antiglucocorticoids (26–28). This property appears to be intimately associated with concomitant changes in the dose-response curve of glucocorticoid agonists. Thus, conditions that cause a shift of the Dex dose-response curve to lower EC_{50}s are always accompanied by an increased partial agonist activity of the antagonists. With the discovery of new antiprogestins with partial PR agonist activity, we wanted to know whether the activity of these compounds could similarly be modified in the presence of different PR concentrations. In preparation for this study, we first inquired whether the dose-response curve of PR agonists could be shifted to lower EC_{50}s in the presence of higher amounts of PR. As shown in Fig. 5, the dose-response curve of PR-agonist complexes is progressively shifted to lower EC_{50}s with higher PR concentrations. The average left shift in the dose-response curve when going from 3 to 30 ng of PR cDNA is 5.2 ± 1.1-fold (SD, n = 5, $P = 0.001$). The *dashed vertical lines* in Fig. 5 show how the activity of a single subsaturating concentration of R5020 varies with different PR concentrations. Thus, as for GR (26–28), the dose-response curve for PR-mediated transactivation is not invariant but can be altered simply by varying the concentration of PR.

Effect of Changing PR Concentration on Dose-Response Curves of New Partial PR Agonists

The precise mechanism by which antisteroids exhibit partial agonist activity is not well understood but is thought to proceed via the same steps as used by full agonists. For this reason, we expected that the magnitude of change in the left shift of the dose-response curve for the PR agonist R5020 with elevated PR (Fig. 5) would be reproduced for the dose-response curves of the partial agonists Dex-Mes and Dex-Ox. Interestingly, this is not the case. With 3 ng of transfected PR-B, the EC_{50} for Dex-Mes is about 350 nM while that for Dex-Ox is a factor of 10 lower at 30 nM (Fig. 6). However, there is only a marginal effect of increased

---

activity was normalized for total protein and plotted as fold induction above the EtOH control (*open bar*). *Error bars* represent SEM (n = 5) for R5020 and Dex-Mes with GREtkCAT and range (n = 2) for all data for PR-A with MMTVLUC. Other data are averages of triplicates from one experiment. B, GREtkLUC reporter in T47D cells. Triplicate cultures were transiently transfected as in panel A with 1 μg of GREtkLUC, treated with either 30 nM R5020 or 1 μM of the designated steroids, and assayed for luciferase activity as described in *Materials and Methods*. The data were normalized for total protein and plotted as fold induction above the EtOH control (*open bar*). *Error bars* indicate the range of two experiments except for RU 486, which is the average of triplicates from one experiment.

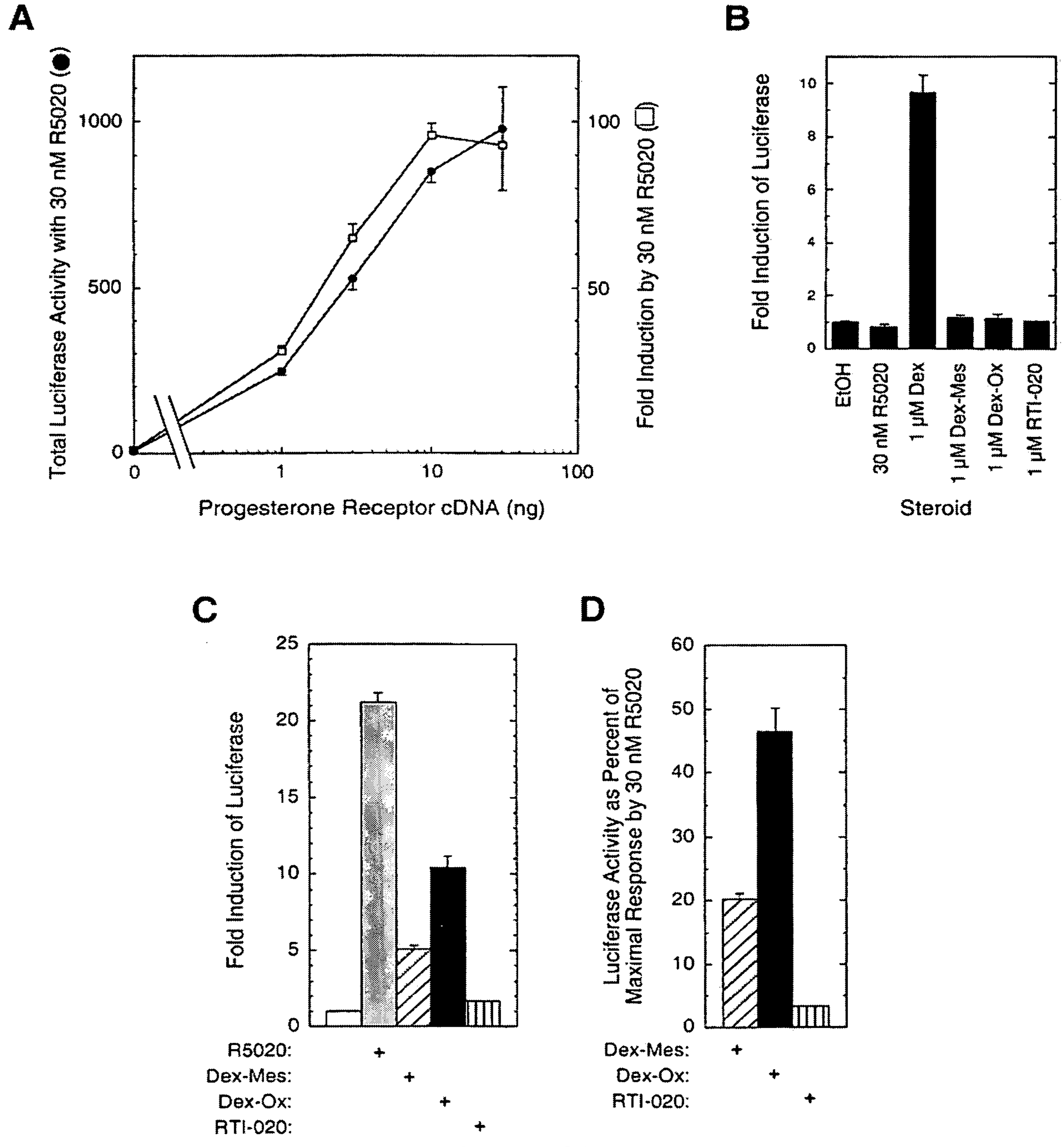

Fig. 3. Gene Activation with Transiently Transfected PR in 1470.2 Cells

A, Effect of PR concentration on total transactivation and fold induction with R5020. Triplicate cultures were transfected with MMTVLuc and *Renilla* null luciferase reporters plus the indicated amounts of hPR-B cDNA plasmid, induced with 30 nM R5020, and assayed for luciferase and *Renilla* activities as described in *Materials and Methods.* The average values, normalized for cotransfected *Renilla*, were then plotted. Similar results were obtained in at least two other experiments. B, Activity of various steroids with endogenous GR of 1470.2 cells. Triplicate cultures were transfected with GREtkLUC reporter as in panel A and induced with the indicated steroids. The levels of luciferase activity, normalized for cotransfected *Renilla*, were then expressed as fold induction above basal levels. Similar results were obtained in a second experiment. C and D, Activity of Dex derivatives with transiently transfected PR in 1470.2 cells. Triplicate cultures were transfected with 3 ng PR cDNA plasmid and GREtkLUC reporter as in panel A and induced with the indicated steroids. The levels of luciferase activity, normalized for cotransfected *Renilla*, were then expressed as fold induction above basal levels in panel C. The same data were replotted in panel D as percent of the maximal induction (30 nM R5020) above basal levels (*open bar* in panel C) to show directly the percent partial agonist activity. The *error bars* in panels C and D indicate the SD of triplicate values. Similar results were obtained in a second experiment.

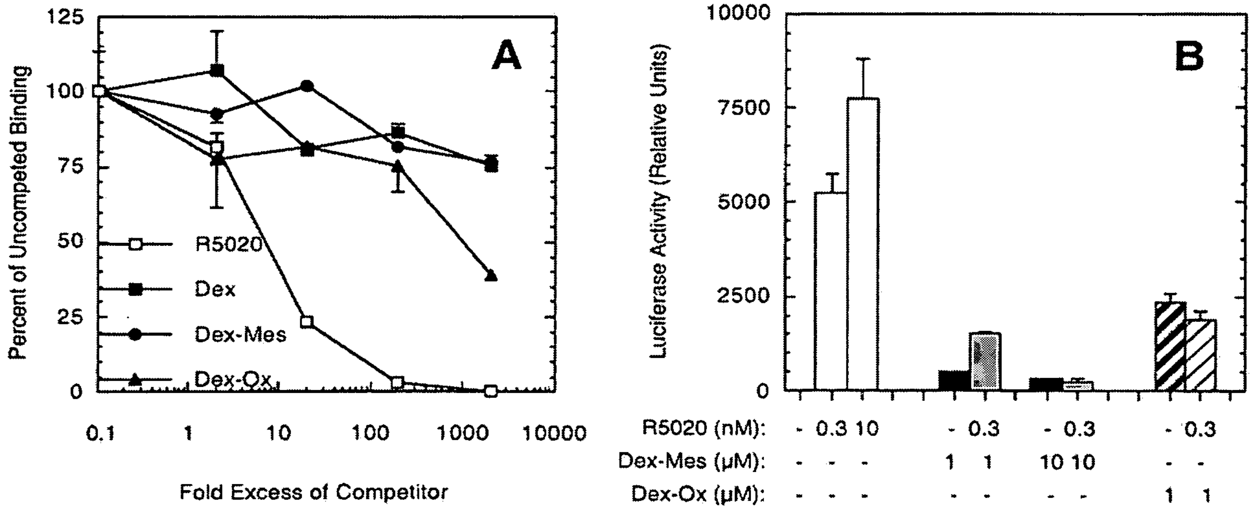

Fig. 4. Antiprogestin Properties of Dex-Mes and Dex-Ox

A, Competition of R5020 binding to cell-free receptors. Duplicate samples of 2.5 nM $[^3H]$R5020 plus the indicated excesses of nonlabeled steroids were assayed for the total binding of $[^3H]$R5020 to cell-free preparations of overexpressed hPR-B at 0 C, as described in *Methods and Materials*. The range of duplicate values is indicated by the *error bars*. Similar results were obtained in a second experiment. B, Competition of PR-mediated induction by R5020 in intact cells. Triplicate cultures were transfected with 3 ng hPR-B cDNA plasmid and 1 μg MMTVLUC as in Fig. 3A and induced with the indicated concentrations of R5020 ± Dex-Mes or Dex-Ox. The absolute levels of luciferase activity, normalized for cotransfected *Renilla* (± SD of triplicate values), were plotted.

PR on the position of the dose-response curve for Dex-Mes (Fig. 6A: 1.49 ± 0.03-fold; SD, $n = 3$, $P = 0.0014$) under conditions where there is a 7-fold left shift of the R5020 dose-response curve (Fig. 6A, *insert*). Similarly, the shift in the dose-response curve for Dex-Ox with added PR is much less (2.6 ± 0.4-fold; $n = 2$; Fig. 6B) than that for R5020 (6.8 ± 1.6-fold, $n = 2$; see *insert*). These differences in the magnitude of left shift of R5020 *vs.* Dex-Mes and Dex-Ox suggest that the mechanistic details by which higher levels of PR can reposition the dose-response curve are not the same for PR-bound agonists *vs.* antagonists/partial agonists.

Effect of Changing PR Concentration on the Partial Agonist Activity of Dex-Mes and Dex-Ox

Although there is little effect of changing PR concentration on the dose-response curves of Dex-Mes or Dex-Ox (Fig. 6), it can not be predicted what the effect will be on the partial agonist activity of each steroid with PR. This is because there is no known relationship between the EC$_{50}$ for steroid induction of a responsive gene and the total level of transactivation for the same gene. To address this question, we performed experiments such as in Fig. 3C to determine the partial activity of Dex-Mes and Dex-Ox at two different PR concentrations. However, for ease of interpretation, the data were replotted as percent of maximal induction by saturating concentrations of R5020, as in Fig. 3D. This is done because the total amount of transactivation by R5020 increases at higher levels of PR (see

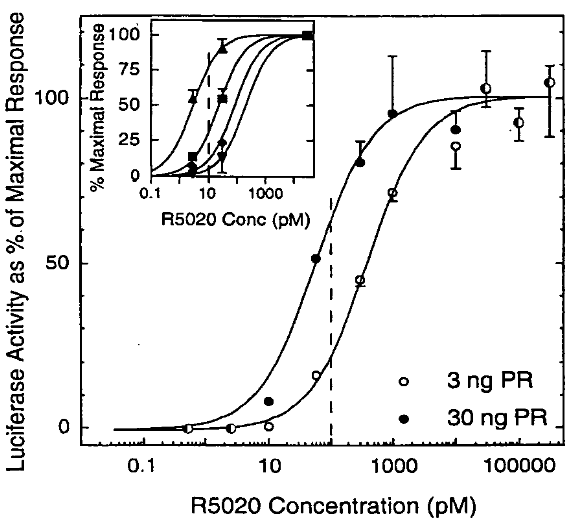

Fig. 5. Effect of PR Concentration on the R5020 Dose-Response Curve and EC$_{50}$

Triplicate cultures were transfected as in Fig. 3A with 3 or 30 ng of hPR-B cDNA plasmid and treated with the indicated concentrations of R5020. The induced values were plotted as percent of the maximal induction with R5020 above basal levels. *Inset* shows the results of an experiment with 0.1 (▼), 1 (◆), 10 (■), and 100 (▲) ng of hPR-B cDNA plasmid (basal levels did not change significantly). The SD of triplicate values is indicated by the *error bars*. The *dashed vertical lines* show how the activity of a single subsaturating concentration of R5020 varies with different PR concentrations. Similar results were obtained in at least two other experiments.

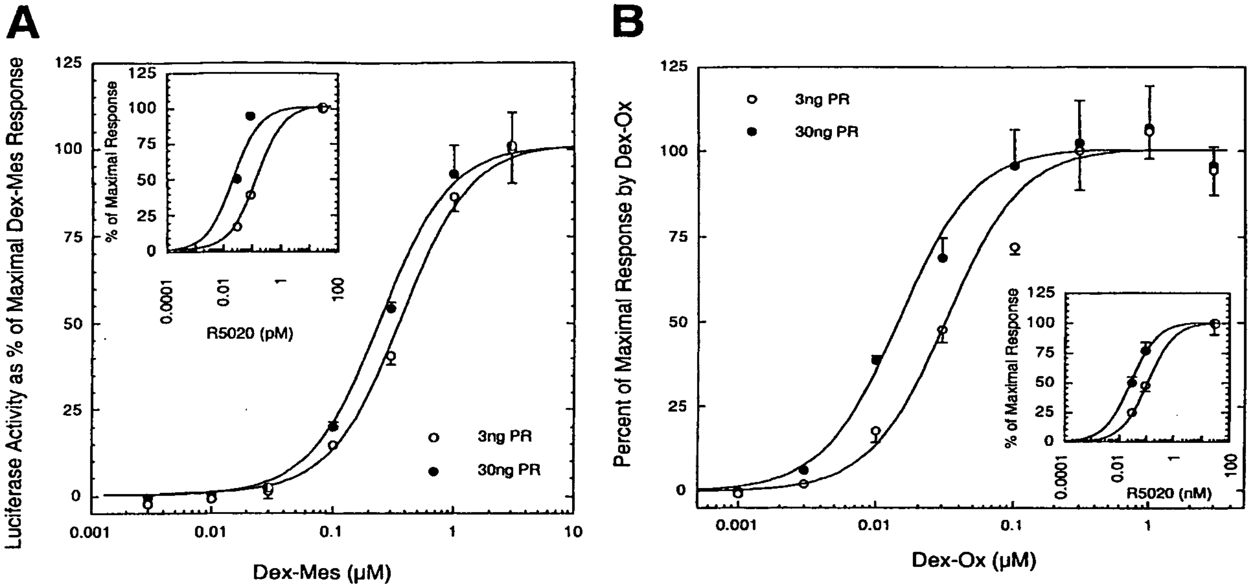

Fig. 6. Effect of PR Concentration on the Dose-Response Curve and EC$_{50}$ of (A) Dex-Mes and (B) Dex-Ox in 1470.2 Cells

After transfecting triplicate cultures with 3 or 30 ng of hPR-B cDNA plasmid as in Fig. 3A, the luciferase values induced by the indicated concentrations of Dex-Mes (A) or Dex-Ox (B) were normalized for cotransfected Renilla and plotted as percent of the maximal values for the same steroid, as in Fig. 5. *Insets* present, for comparison, the dose-response curves for R5020 with the different PR concentrations in the same experiment. The SD of triplicate values is indicated by the *error bars*. Similar results were obtained in at least one additional experiment.

Fig. 3A), thus making it difficult to directly assess changes in percent agonist activity simply by looking at the raw data. When the results are expressed as percent of maximal induction by R5020, however, it is clear that added PR has a dramatic effect (Fig. 7). The increased activity of subsaturating concentrations of R5020 (30 and 90 pM) is what is expected from a left shift in the dose-response curve for R5020 (see Fig. 5). For Dex-Mes and Dex-Ox, conditions that have little effect on the dose-response curves of these steroids (see Fig. 6) have a very large effect on the partial agonist activity relative to saturating concentrations of R5020. Dex-Mes has about 20% activity with 3 ng PR but 60% activity with 30 ng PR. Dex-Ox, which displays around 50% agonist activity with 3 ng PR, is almost a full agonist with 30 ng PR. These dramatic increases in partial agonist activity can occur in the absence of appreciable changes in EC$_{50}$ because they depend upon different parameters. The partial agonist activity of the antiprogestins is determined by the total transactivation with 1 μM antagonist as a percent of the total transactivation by saturating concentrations of R5020 at each PR concentration. On the other hand, the EC$_{50}$ for the antagonist with 3 and 30 ng PR is independent of the activity of R5020 or the absolute amount of transactivation by 1 μM antagonist. In summary, it is clear that the partial agonist activity of Dex-Mes and of Dex-Ox with PR is not constant but increases in proportion to the amount of PR present in the cell.

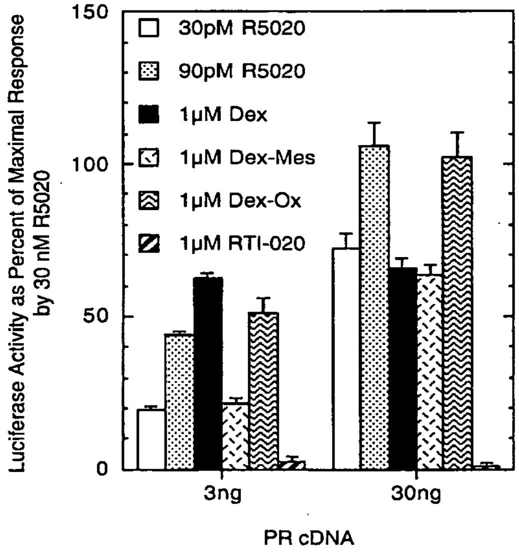

Fig. 7. Variation of Partial Agonist Activity of Antiprogestins with Increasing PR in 1470.2 Cells

Triplicate cultures were transfected with 3 or 30 ng of PR cDNA and 1 μg MMTVLUC as in Fig. 3A, followed by treatment with the indicated steroids. The induced luciferase values were determined, normalized for cotransfected *Renilla*, and plotted as percent of maximal induction by 30 nM R5020, as in Fig. 3D. The SD of triplicate values is indicated by the *error bars*. Similar results were obtained in a second experiment.

It is interesting that the partial agonist activity of RTI-020 does not increase under conditions that augment the activity of Dex-Mes and Dex-Ox (Fig. 7). Thus, while RTI is a partial agonist under some conditions (16), Dex-Mes and Dex-Ox retain partial agonist activity under a possibly wider variety of conditions.

Effects of Coactivators and Corepressors on PR Induction Properties

The total transactivation of responsive genes by PR has been found to increase in the presence of cotransfected coactivators (43, 44) while corepressors can decrease receptor transactivation (8, 18, 19, 26). Recently, coactivators and corepressors have also been shown to modify the dose-response curve of GR-agonist complexes and the partial agonist activity of GR-antagonist complexes (26, 27). Given the high homology between GR and PR, we asked whether similar effects might be seen with PR complexes. Such a study was difficult before our discovery of the partial agonist activities of Dex-Mes and Dex-Ox because neither of the existing antiprogestins, RU 486 or RTI-020, displays appreciable agonist activity (Figs. 2, 3, and 7).

When TIF2 is cotransfected with PR in 1470.2 cells, the total transactivation increases about 25%, as expected for a coactivator. At the same time, a weak but statistically significant shift to lower steroid concentrations is seen in the dose-response curve [Fig. 8A: 1.95 ± 0.53 fold (SD), n = 4, $P = 0.038$]. At the same time, added TIF2 enhances the partial agonist activity of Dex-Mes (Fig. 8B). Interestingly, while the effect of added TIF2 is not as great as added PR on the positioning of the dose-response curve for R5020 (Fig. 8A), TIF2 is equally effective in increasing the partial agonist activity of Dex-Mes (Fig. 8B). This difference in sensitivity to TIF2, relative to increased PR, suggests that the mechanisms involved in TIF2 modulation of agonist and antagonist induction properties are not the same.

It should be noted that TIF2 had no effect on the induction properties of either PR or GR in T47D cells under conditions where increased GR did have an effect on the Dex EC_{50} (data not shown). Thus, it appears that the ability of TIF2 to modulate PR, or GR, induction properties can be cell specific.

SRC-1 (steroid receptor coactivator 1) is another coactivator that is closely related to TIF2 (45, 46) and thus might be expected to have similar effects as TIF2. To examine the effects of a range of SRC-1 concentrations, we did not perform an entire dose-response curve but rather assayed the percent of maximal activity of just a single subsaturating concentration of R5020. As shown in Fig. 5 (*dashed lines*), this abbreviated assay is a valid test for changes in the R5020 dose-response curve or EC_{50}. Surprisingly, additions of up to 1.8 μg of SRC-1 have no significant effect on either the EC_{50} for R5020, as indicated by the activity of a subsaturating concentration of R5020 (Fig. 9), or the total amount of transactivation (data not shown). Similarly, neither CREB-binding protein (CBP) nor 0.45 μg SRC-1 plus 0.2 μg CBP had any effect on the EC_{50} for R5020 or the total amount of transactivation (data not shown). This absence of effect was not due to a

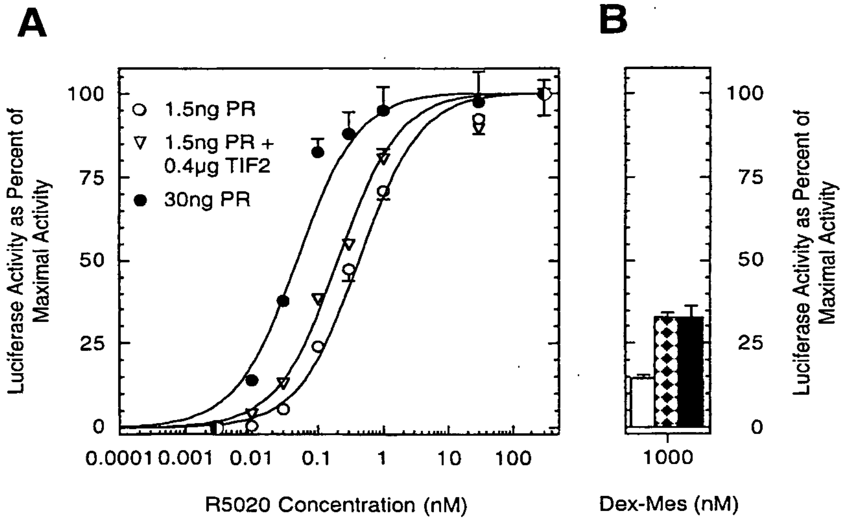

Fig. 8. Modification of Induction Properties of PR-Agonist and Antagonist Complexes in 1470.2 Cells by Added Coactivator TIF2

Triplicate cultures were transfected as in Fig. 3A with 1.5 ng PR ± 0.4 μg TIF2, or 30 ng PR, plus the indicated concentrations of R5020 or 1 μM Dex-Mes. The data for R5020 (A) and Dex-Mes (B) are normalized for cotransfected Renilla and plotted as percent of the maximal values for R5020, as in Fig. 3D. The treatments in panel B are 1.5 ng PR (*open bar*), 1.5 ng PR + 0.4 μg TIF2 (*patterned bar*), and 30 ng PR (*solid bar*). The SD of triplicate values is indicated by the *error bars*. Similar results were obtained in three additional experiments.

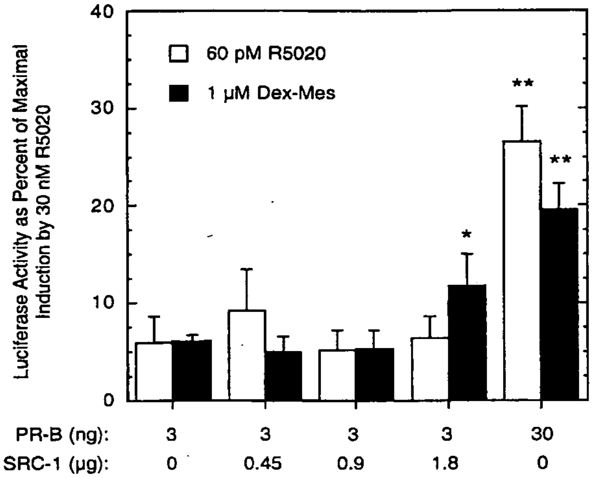

Fig. 9. Influence of Coactivator SRC-1 on Induction Properties of PR-Agonist and Antagonist Complexes in 1470.2 Cells

Triplicate cultures were transfected as in Fig. 3A with PR ± varying amounts of SRC-1, as indicated, and then treated with vehicle ± 60 pM R5020, 30 nM R5020, or 1 µM Dex-Mes. The induction by 60 pM R5020, or 1 µM Dex-Mes, was normalized for cotransfected Renilla and plotted as percent of the maximal activity with 30 nM R5020, as in Fig. 3D. The SD of triplicate values is indicated by the *error bars* (*, $P = 0.04$; **, $P = 0.001$). Similar results were obtained in two additional experiments.

total inactivity of the SRC-1 because 1.8 µg of SRC-1 did increase the partial agonist activity of Dex-Mes with 3 ng of PR to about 65% of that seen with 30 ng of PR (n = 3; $P = 0.048$; see also Fig. 9). Also, the same SRC-1 and CBP plasmid preparations elevated the percent of maximal activities of 1 nM Dex and 1 µM Dex-Mes with GR in CV-1 cells (data not shown and Ref. 26). Therefore, it appears that not all coactivators have the same effects on the activities of PR complexes in 1470.2 cells.

In contrast to coactivators, corepressors, such as SMRT (19) and NCoR (18), typically produce the opposite effects of coactivators. They can decrease the total transactivation of receptors (8, 18, 19, 26) and reduce the partial agonist activity of antisteroids (8, 9, 15, 17, 25, 26). It was therefore of interest to determine what effect increased concentrations of corepressor would have on PR-agonist and partial agonist complexes. Cotransfection of the corepressor SMRT causes a reproducible 2.6 ± 0.4-fold (n = 2) right shift in the dose-response curve (Fig. 10A), and decreased the partial agonist activity of Dex-Mes from 11.1 ± 1.6% to −0.4 ± 1.6% (SD, n = 4; $P < 0.0001$, Fig. 10B), while simultaneously acting as a corepressor to decrease the levels of total transactivation by 13 ± 6% (SEM, n = 5, $P = 0.09$).

The addition of NCoR, another corepressor (18), also suppresses the total transactivation, this time by 28 ± 9% (SEM, n = 9, $P = 0.01$). However, NCoR causes an increase in the activity of subsaturating concentrations of R5020 to shift the dose-response curve to lower EC_{50}s (Fig. 11A). At the same time, added NCoR increased the partial agonist activity of saturating concentrations of Dex-Mes (Fig. 11B; averages values = 36 ± 17% without NCoR *vs.* 69 ± 16% with NCoR, n = 6, SD, $P = 0.0064$). Therefore, both SMRT and NCoR appear to act as corepressors by decreasing the total transactivation, but they elicit almost exactly opposite behaviors in the induction properties of PR complexes.

DISCUSSION

This study describes evidence that Dex-Mes and Dex-Ox, two C-17 position derivatives of the GR-selective ligand Dex, are new antiprogestins with partial agonist activity. These steroids are active with PR-A and -B isoforms, different reporters [CAT (chloramphenicol acetyltransferase) and LUC (luciferase)], and different promoters and enhancers (GREtk and MMTV) in a variety of cells (CV-1, 1470.2, and T47D). To the best of our knowledge, Dex-Mes is the first compound to exhibit partial agonist activity with PR-A (Fig. 2A) (14, 47–49). The high level of agonist activity of Dex-Ox with the endogenous PR of T47D cells, which is predominantly PR-A (50), suggests that Dex-Ox, like Dex-Mes, also possesses agonist activity with PR-A receptors. The data with T47D cells further demonstrate that the activities of Dex-Ox in CV-1 and 1470.2 cells are not an artifact of transient transfection. This, in turn, indicates that the partial agonist activity of Dex-Mes with PR is also not limited to transfected PR.

With any new steroid, an obvious question is whether the observed biological activities result from the formation of receptor-steroid complexes. Five lines of evidence indicate that the effects of both Dex-Mes and Dex-Ox are mediated by direct interactions with PR. First, the partial agonist activity of both steroids in 1470.2 (Fig. 3, B and C) and CV-1 (data not shown) cells is dependent upon transfected PR. Second, cell-free competitive binding studies show that Dex-Ox binds PR-B with an affinity that is 1% of the potent progestin R5020 and suggest that the affinity of Dex-Mes is 0.1% of R5020 (Fig. 4A). Third, the relative concentrations of R5020, Dex-Ox, and Dex-Mes required for 50% inhibition in the cell-free binding studies (1:100:1,000, respectively) correspond closely to the biological potencies of each steroid as an agonist, as determined from the dose-response curves with 3 ng of PR-B cDNA in Figs. 5 and 6 (EC_{50}s: R5020, 0.35 nM; Dex-Ox, 30 nM; and Dex-Mes, 350 nM). Fourth, the 10-fold greater potency of Dex-Ox *vs.* Dex-Mes as an antiprogestin (Fig. 4B) correlates with the 10-fold higher apparent affinity of Dex-Ox for PR. Fifth, the low activity of Dex-Mes in intact cells can be partially reversed by added R5020 (Fig. 4B), which argues that at least some of the effects of Dex-Mes are mediated by specific interactions with PR. Collectively, we feel

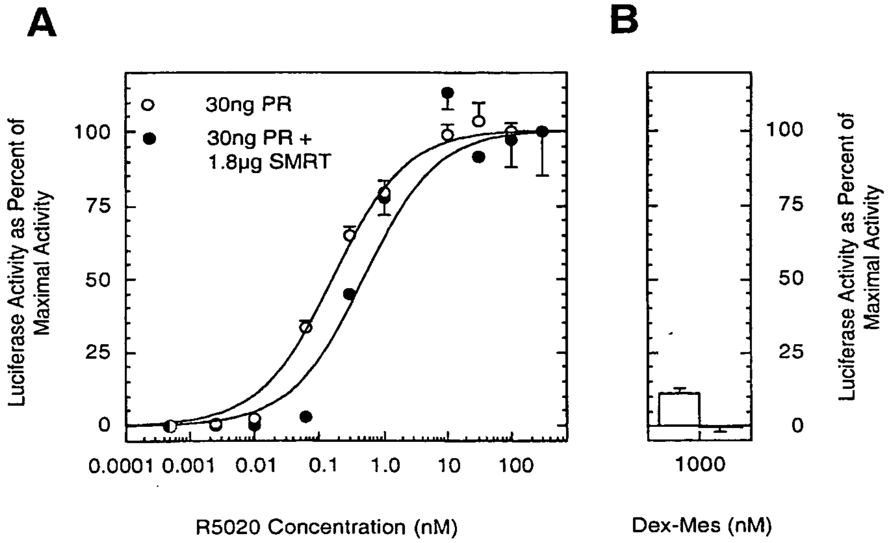

Fig. 10. Effect of Added Corepressor SMRT on Induction Properties of PR-Agonist and Antagonist Complexes in 1470.2 Cells

Triplicate cultures were transfected as in Fig. 3A with 30 ng PR ± 1.8 μg SMRT plus the indicated concentrations of R5020 or 1 μM Dex-Mes. The data are normalized for cotransfected *Renilla* and plotted as percent of the maximal values for R5020 for (A) one of two experiments with R5020 and for (B) the average (±SD) of four experiments with Dex-Mes, as in Fig. 3D.

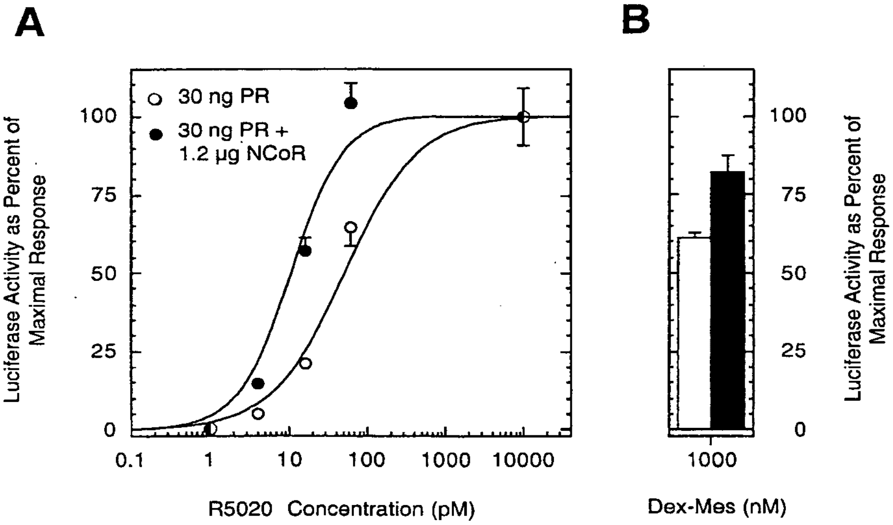

Fig. 11. Modulation of Induction Properties of PR-Agonist and Antagonist Complexes in 1470.2 Cells by Added Corepressor NCoR

Triplicate cultures were transfected as in Fig. 3A with 30 ng PR ± 1.2 μg NCoR plus the indicated concentrations of R5020 or 1 μM Dex-Mes. The data for R5020 (A) and Dex-Mes (B) are normalized for cotransfected Renilla and plotted as percent of the maximal values for R5020, as in Fig. 3D. The SD of triplicate values is indicated by the *error bars*. Similar results were obtained in two (A) or five (B) additional experiments.

these data strongly support the conclusion that Dex-Mes and Dex-Ox are two new antiprogestins that display significant amounts of partial agonist activity under a variety of conditions due to their binding to PR.

Very few partial agonists for PR exist in the literature. The most active such compound to be reported is RTI-020 and some closely related derivatives (16), which are structurally similar to the almost pure antiprogestin RU 486 (Fig. 1). Interestingly, RTI-020 is inactive in CV-1 (16) and 1470.2 cells, even under conditions where Dex-Mes and Dex-Ox are active (Figs. 2A and 3). These data suggest that D-ring derivatives at the C-17 position of Dex (Fig. 1) may be a rich new source of future antiprogestins with partial agonist activity. As the oxetanone modification of Dex (Fig. 1) increased the apparent affinity for PR by about a factor of 10 (Fig. 4A), it is reasonable to expect that other derivatives with even higher affinity for PR might be found to retain appreciable amounts of partial PR agonist activity.

The discovery of two new antiprogestins that possess partial agonist activity (Dex-Mes and Dex-Ox) facilitated our subsequent studies of whether the induction properties of PR can be modified by changing the receptor concentration, as has recently been described for GR (26–28). Thus, in addition to looking at the ability of variations in PR level to alter the position of the dose-response curve of agonists (or EC_{50}), we could now determine whether this is accompanied by coordinated changes in the partial agonist activity of antiprogestins. The present results demonstrate for the first time that both properties of PR gene induction are influenced by PR concentration. The fact that PR concentration is not constant among PR-responsive tissues leads to the additional prediction that the partial agonist activity of Dex-Mes and Dex-Ox will also not be constant in all tissues. Furthermore, the level of PR in a given cell can be modified, as seen in breast cells where estrogens usually induce PR levels (51). Therefore, even in the same cell, the partial agonist activity of Dex-Mes or Dex-Ox may change.

Another modulator of the partial agonist activity of antiprogestins is shown here to be coregulators. Elevated levels of the coactivators TIF2 and SRC-1, along with the corepressor NCoR, can each augment the partial agonist activity of Dex-Mes. As the levels of these coregulators are not uniform among cells (30–32), or even in a given cell (25, 30), it is likely that partial agonist activity of Dex-Mes will vary from cell to cell. Furthermore, the capacity of PR to respond to added TIF2 is cell-dependent, as seen here by the effects on PR in 1470.2 cells but not in T47D cells. This suggests that the influence of PR and coregulator concentrations on the partial agonist activity of Dex-Mes, or Dex-Ox, will be additionally modified by other cell-specific factors. This combinatorial diversity makes Dex-Mes and Dex-Ox promising candidates as new SPRMs that could exhibit varying amounts of partial agonist activity for a given gene among diverse cells and potentially even different genes within a given cell. It should also be noted that these same variables have been found to influence the dose-response curve (or EC_{50}) of PR-agonist complexes (Figs. 1 and 5) and thus could afford differential control of transcription of responsive genes in different cells by modifying the sensitivity to subsaturating concentrations of progestins and possibly of different genes within the same cell.

The absence of any effect of SRC-1 on the EC_{50} or total transactivation of PR-agonist complexes in 1470.2 cells was unexpected as SRC-1 was first discovered for its ability to increase the transcriptional activity of PR (43). Similarly, we did not observe the synergistic increase in the levels of PR-mediated transactivation reported for the combination of SRC-1 and CBP (52). It is important to realize that SRC-1 was not totally inactive in our system but did augment the partial agonist activity of the antiprogestin Dex-Mes. Therefore, the simplest explanation for these different responses of PR to SRC-1 is that they reflect tissue-specific variations. Almost all reports of SRC-1 activity with PR have been in HeLa cells (43, 44, 53) and none have appeared for 1470.2 cells. Similar examples of cell-specific activities of cofactors have been described (26, 45), and seen here, with TIF2. Whether this reflects different levels of endogenous coactivators (25, 26, 30, 32, 33), or a more basic selectivity in the effects of various coactivators on PR induction parameters, remains to be established.

TIF2/GRIP1 is reported not to interact with PR complexed with RU 486 (45, 46). However, with the new antiprogestin Dex-Mes, there is a clear effect of added TIF2 on the PR complexes (Fig. 8), consistent with a functional interaction. This suggests that the conformation of the Dex-Mes complex is different from that with RU 486 and permits associations with both coactivators and corepressors. It is tempting to speculate that the smaller size of Dex-Mes *vs.* RU 486 (54) permits an equilibrium mixture of a wider variety of tertiary structures of the ligand-bound PR, with different structures having modified affinities for coactivators or corepressors. The magnitude of conformational changes need not be dramatic because coactivators and corepressors can both bind to the same general hydrophobic pocket of steroid/nuclear receptors (20, 21, 24).

The observed effects of added SMRT and NCoR (Figs. 10 and 11) indicate a functional interaction of corepressors with both PR-agonist and PR-antagonist complexes. SMRT and NCoR both act as corepressors in 1470.2 cells in that they suppress the total levels of PR transactivation but have exactly opposite effects on other induction parameters (Figs. 10 and 11). SMRT causes a right shift of the R5020 dose-response curve to higher EC_{50}s while NCoR induces a left shift. The partial agonist activity of the antisteroid Dex-Mes is decreased by SMRT but increased by NCoR. In many respects, NCoR acts more like the coactivator TIF2. While part of the explanation of these divergent results may be yet another example of cell-specific differences, they complicate a clear-cut distinction between coactivators and corepressors. These differences between NCoR and SMRT are reminiscent of the reports that basal transcription by TR is increased by added SMRT but not NCoR (55) and that DAX-1 (56) and RevErb (57) display differential interactions for SMRT and NCoR.

We have recently reported analogous effects of receptor and coregulator concentrations on GR induction properties (26). These results led to a model in which the EC_{50}, and partial agonist activity, of transcriptionally active GR complexes is influenced by a dynamic equilibrium of receptor and coregulator proteins (26). More recent studies (27) indicate that the effects of varying concentrations of both GR and coactivators are manifested via a shared mechanistic step (X in Fig. 12). We suspect that a similar common intermediate is involved in the modulation of PR complex activities with changing PR and coregulator concentrations. However, this intermediate is unlikely to be the same as that for GR (*i.e.* different from X in Fig. 12) because overexpressed PR does not alter the GR dose-response curve or partial agonist activity of antiglucocorticoids (28). Therefore, the proposed common intermediate for PR action would be either downstream of X or in a different pathway (Fig. 12). The identity of these proposed intermediates is clearly of interest. Unfortunately, very little is known about the role of individual cofactors at any stage of receptor-mediated transactivation.

The effects of added PR on the EC_{50} are consistently much more pronounced for agonists than for antagonists (Fig. 6, A and B). Conversely, the effects of TIF2 and SRC-1 on the partial agonist activity of Dex-Mes are more than on the EC_{50} for R5020 (Figs. 8 and 9). This suggests that the mechanistic details for the modification of PR induction parameters are not the same for agonist and partial agonist complexes. This is not surprising, given the different x-ray structures of receptor ligand-binding domains with agonist *vs.* antagonist steroids (58, 59). Whether this structural diversity is translated into altered affinities for common cofactors or very different affinities for completely separate cofactors remains to be determined.

Dex-Mes and Dex-Ox are also antiglucocorticoids (34, 35). While they evoke negligible agonist activity through the endogenous GR of 1470.2 cells (Figs. 3A and 4A), they display significant amounts of glucocorticoid activity in other cells (26, 28, 34, 35, 39). This is in contrast to the most commonly used antiprogestin, RU 486, which is a pure antiglucocorticoid in a wide variety of cells. Therefore, the use of Dex-Mes and

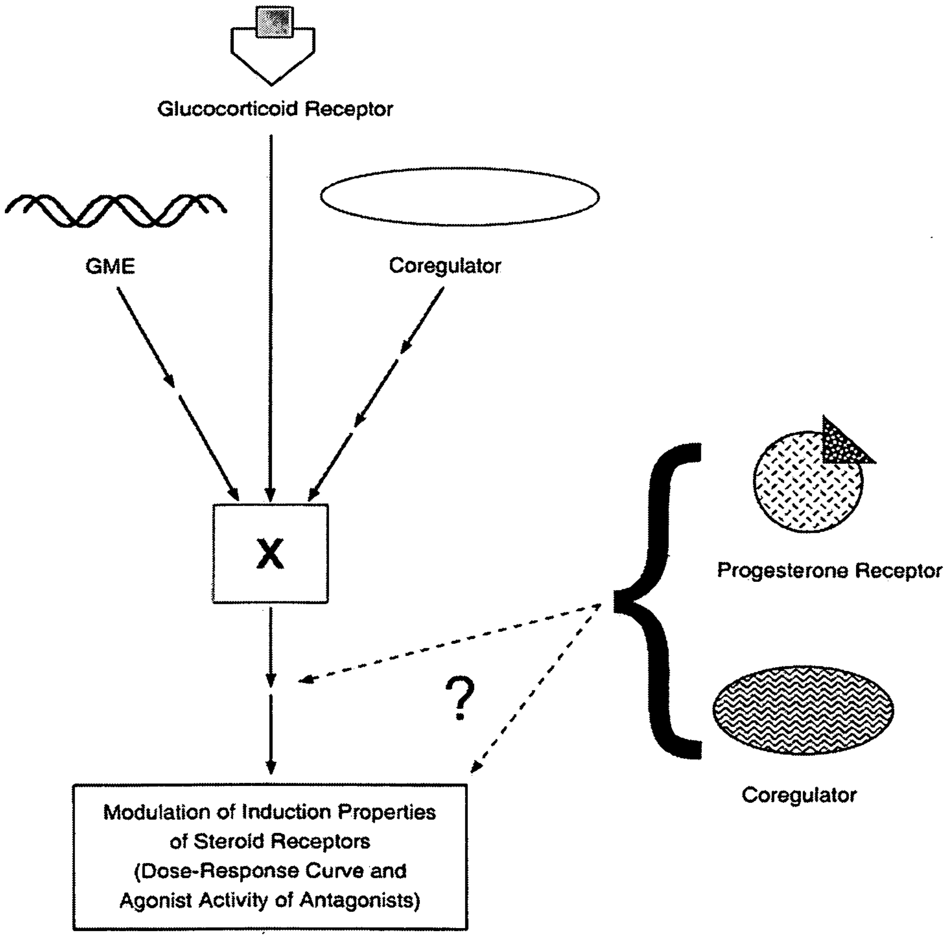
Glucocorticoid Receptor
GME
Coregulator
X
Progesterone Receptor
?
Coregulator
Modulation of Induction Properties of Steroid Receptors (Dose-Response Curve and Agonist Activity of Antagonists)

Fig. 12. Proposed Model by Which Added PR, and Increasing Concentrations of Coregulators, Modify the Dose-Response Curve of PR-Agonist Complexes and the Partial Agonist Activity of PR-Antagonist Complexes

Both GR and PR are capable of inducing the same reporter gene, but the components that can alter the induction properties of PR (PR and coregulator concentrations) are speculated to affect a different step or pathway than for GR, where several components (glucocorticoid modulatory element, GR concentration, and coregulator concentration) have been found to competitively interact at a step labeled X (27). See text for further details.

Dex-Ox as antiprogestins should be accompanied by reduced side effects since fewer actions of GRs will also be blocked. Dex-Mes is an electrophilic affinity label but does not covalently label PR (60, 61). While the chemical reactivity of Dex-Mes, and reaction with a variety of proteins, is not a problem when used to examine PR- or GR-mediated responses in tissue culture cells, it probably limits the applications in intact animals and humans (62). Dex-Ox, however, is not subject to any such restrictions and may find more widespread applications.

In conclusion, alterations in the intracellular levels of PR, coactivators, and corepressors can affect two physiologically relevant induction properties of PR: the partial agonist activity of some antiprogestins and the EC_{50} of PR agonists. Cellular variations in the level of coactivators and corepressors (25, 29–33) are common. When this effect is superimposed on the alterations in PR levels, especially in response to induction by estrogen receptors (51), the differences in the transactivation of target gene in various cells with both subsaturating concentrations of agonist and saturating concentrations of antagonist may be substantial. Given the many mechanistic similarities of all of the steroid receptors, it will be interesting to test the hypothesis that the induction properties of androgen, estrogen, and mineralocorticoid receptors can be similarly modified.

MATERIALS AND METHODS

Unless otherwise indicated, all operations were performed at 0 C.

Chemicals, Buffers, and Plasmids

[17α-methyl-^{3}H]Promegestone (R5020, 85Ci/mmol) and nonradioactive R5020 were obtained from NEN Life Science Products (Boston, MA) and dexamethasone (Dex) from Sigma (St. Louis, MO). Dex-Ox (63) and Dex-Mes (64) were prepared as described, and RTI-3021–020 (RTI-020) was a gift from C. Edgar Cook (Research Triangle Institute, Research Triangle Park, NC). Restriction enzymes and digestions were performed according to the manufacturer's specifications (New England Biolabs, Inc., Beverly, MA).

MMTVLuc (pLTRLuc) was provided by Gordon Hager (NIH, Bethesda, MD). The *Renilla* null luciferase reporter was purchased from Promega Corp. (Madison, WI). The cDNA plasmids of SRC-1a (Bert O'Malley, Baylor College of Medicine, Houston, TX), TIF2 and A and B forms of human PR (Hinrich Gronemeyer, IGBMC, Strasbourg, France), CBP (Richard Goodman, Vollum Institute, Portland, OR), NCoR (Michael Rosenfeld, University of California, San Diego), and SMRT (Ron Evans, Salk Institute, La Jolla, CA) were each received as gifts.

Cell Culture and Transfection

Monolayer cultures of COS-7 and 1470.2 cells were grown at 37 C with 5% CO_2 in DMEM (Life Technologies, Inc., Gaithersburg, MD) and DMEM with 4.5 g glucose/liter (Quality Biologicals, Inc., Gaithersburg, MD), respectively, supplemented with 5% and 10% of FBS, respectively. CV-1 (28) and T47D (50) cells were grown as described. Coregulator plasmids were transiently cotransfected into 1470.2 cells using calcium phosphate with the human PR-B (hPR-B) or hPR-A construct, 1 μg of MMTVLuc (or 2 μg of GREtkCAT or GREtkLuc), and 50 ng *Renilla* null luciferase, with the total transfected DNA brought up to 3 μg/60-mm dish with pBSK$^+$ DNA (28). In experiments with coregulator cDNA plasmid, all cells not transfected with coregulator were cotransfected with an equimolar amount of the same plasmid vector to control for artifacts of the vector DNA. The cells were treated for 24 h with steroids in media containing 10% charcoal-stripped FBS (HyClone Laboratories, Inc. Logan, UT) (regular FBS for other cells) and harvested in 1× Passive Lysis Buffer (0.5 ml/dish, Promega Corp.). Twenty microliters of the cell lysates were used to assay for luciferase activity using the Dual-Luciferase Assay System from Promega Corp. according to instructions of the supplier. The data were then normalized either for total protein or cotransfected *Renilla* activity.

Assay for Steroid Competition

Transient transfection of COS-7 cells with 10 μg/10-cm plate of human PR-B cDNA was performed as described previously (65). Cytosols of transfected cells containing the steroid-free receptors were obtained by the lysis of cells at −80 C and centrifugation at 15,000 × *g* (66). Thirty percent cytosol with 20 mM sodium molybdate was adjusted to 2.5 nM [^{3}H]R5020 ± varying amounts of nonradioactive competitor and incubated at 0 C for 18 h. Unbound [^{3}H]R5020 was removed by dextran-coated charcoal. The specific binding, calculated by subtracting the background disintegrations/min (2,000-fold R5020) from the total [^{3}H]R5020 binding in the presence or absence of cold competitor, was divided by the specific disintegrations/min of the uncompeted [^{3}H]R5020 and expressed as the percent of uncompeted binding.

Analysis of Data

The activity for subsaturating concentrations of agonist, or saturating concentrations of antagonist, was expressed as percent of maximal activity with saturating concentrations of agonist (30 nM R5020 unless otherwise noted). The fold induction with 30 nM R5020 was calculated as the luciferase activity (relative firefly light units/relative *Renilla* light units) activity with 30 nM R5020 divided by the basal activity obtained with ethanol. Individual values were generally within ± 20% of the average, which was plotted. Unless otherwise noted, all statistical analyses were by two-tailed Student's *t* test using the program InStat 2.03 for Macintosh (GraphPad Software, Inc., San Diego, CA).

Acknowledgments

We thank Edgar Cook, Ron Evans, Richard Goodman, Hinrich Gronemeyer, Gordon Hager, Bert O'Malley, and Michael Rosenfeld for the gift of research materials and George Chrousos (NIH) for critical review of the paper.

Received August 22, 2000. Revision received October 12, 2000. Accepted October 30, 2000.

Address requests for reprints to: Dr. S. Stoney Simons, Jr., Building 8, Room B2A-07, NIDDK/LMCB, NIH, Bethesda, Maryland 20892-0805.

*Current address: Sequenom, 142-F North Road, Suite 150, Sudbury, Massachusetts 01776.

†Current address: 306 Henderson Street, Chapel Hill, North Carolina 27514.

REFERENCES

1. Baulieu EE 1989 Contragestion and other clinical applications of RU 486, an antiprogesterone at the receptor. Science 245:1351–1357
2. Spitz IM, Bardin CW 1993 Mifepristone (RU 486)–a modulator of progestin and glucocorticoid action. N Engl J Med 329:404–412
3. Jordan VC, Murphy CS 1990 Endocrine pharmacology of antiestrogens as antitumor agents. Endocr Rev 11: 578–610
4. Stuart NS, Warwick J, Blackledge GR, Spooner D, Keen C, Taylor AR, Tyrell C, Webster DJ, Earl H 1996 A randomised phase III cross-over study of tamoxifen versus megestrol acetate in advanced and recurrent breast cancer. Eur J Cancer 32A:1888–1892
5. Miller PA, Ostrowski MC, Hager GL, Simons Jr SS 1984 Covalent and noncovalent receptor-glucocorticoid complexes preferentially bind to the same regions of the long terminal repeat of murine mammary tumor virus proviral DNA. Biochemistry 23:6883–6889
6. Sistare FD, Hager GL, Simons Jr SS 1987 Mechanism of dexamethasone 21-mesylate antiglucocorticoid action: I. Receptor-antiglucocorticoid complexes do not competitively inhibit receptor-glucocorticoid complex activation of gene transcription *in vivo*. Mol Endocrinol 1:648–658
7. Horwitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GS, Tung L 1996 Nuclear receptor coactivators and corepressors. Mol Endocrinol 10:1167–1177
8. Smith CL, Nawaz Z, O'Malley BW 1997 Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11:657–666
9. Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen T-M, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW 1998 Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. Proc Natl Acad Sci USA 95:2920–2925
10. Mahajan DK, London SN 1997 Mifepristone (RU486): a review. Fertil Steril 68:967–976
11. Wehle H, Moll J, Cato ACB 1995 Molecular identification of steroid analogs with dissociated antiprogestin activities. Steroids 60:368–374
12. Yang NN, Venugopalan M, Hardikar S, Glasebrook A 1996 Identification of an estrogen response element activated by metabolites of 17β-estradiol and raloxifene. Science 273:1222–1225
13. Gradishar WJ, Jordan VC 1997 Clinical potential of new antiestrogens. J Clin Oncol 15:840–852
14. Meyer M-E, Pornon A, Ji J, Bocquel M-T, Chambon P, Gronemeyer H 1990 Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. EMBO J 9:3923–3932
15. Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB 1997 The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11:693–705
16. Wagner BL, Pollio G, Leonhardt S, Wani MC, Lee DY-W, Imhof MO, Edwards DP, Cook CE, McDonnell DP 1996 16α-Substituted analogs of the antiprogestin RU486 induce a unique conformation in the human progesterone receptor resulting in mixed agonist activity. Proc Natl Acad Sci USA 93:8739–8744
17. Wagner BL, Norris JD, Knotts TA, Weigel NL, McDonnell DP 1998 The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. Mol Cell Biol 18:1369–1378
18. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, Rosenfeld MG 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397–404
19. Chen JD, Evans RM 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454–457
20. Hu X, Lazar MA 1999 The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. Nature 402:93–96
21. Nagy L, Kao H-Y, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JWR 1999 Mechanism of corepressor binding and release from nuclear hormone receptors. Genes Dev 13:3209–3216
22. Glass CK, Rosenfeld MG 2000 The coregulator exchange in transcription functions of nuclear receptors. Genes Dev 14:121–141
23. McKenna NJ, Lanz RB, O'Malley BW 1999 Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321–344
24. Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krones A, Rose DW, Lambert MH, Milburn MV, Glass CK, Rosenfeld MG 1999 Molecular determinants of nuclear receptor-corepressor interaction. Genes Dev 13: 3198–3208
25. Soderstrom M, Vo A, Heinzel T, Lavinsky RM, Yang W-M, Seto E, Peterson DA, Rosenfeld MG, Glass CK 1997 Differential effects of nuclear receptor corepressor (N-CoR) expression levels on retinoic acid receptor-mediated repression support the existence of dynamically regulated corepressor complexes. Mol Endocrinol 11: 682–692
26. Szapary D, Huang Y, Simons Jr SS 1999 Opposing effects of corepressor and coactivators in determining the dose-response curve of agonists, and residual agonist activity of antagonists, for glucocorticoid receptor regulated gene expression. Mol Endocrinol 13:2108–2121
27. Chen S, Sarlis NJ, Simons Jr SS 2000 Evidence for a common step in three different processes for modulating the kinetic properties of glucocorticoid receptor-induced gene transcription. J Biol Chem 275:30106–30117
28. Szapary D, Xu M, Simons Jr SS 1996 Induction properties of a transiently transfected glucocorticoid-responsive gene vary with glucocorticoid receptor concentration. J Biol Chem 271:30576–30582
29. Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965–968
30. Misiti S, Schomburg L, Yen PM, Chin WW 1998 Expression and hormonal regulation of coactivator and corepressor genes. Endocrinology 139:2493–2500
31. Suen C-S, Berrodin TJ, Mastroeni R, Cheskis BJ, Lyttle CR, Frail DE 1998 A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. J Biol Chem 273: 27645–27653
32. Shim W-S, DiRenzo J, DeCaprio JA, Santen RJ, Brown M, Jeng M-H 1999 Segregation of steroid receptor coactivator-1 from steroid receptors in mammary epithelium. Proc Natl Acad Sci USA 96:208–213
33. Folkers GE, van der Burg B, van der Saag PT 1998 Promoter architecture, cofactors, and orphan receptors contribute to cell-specific activation of the retinoic acid receptor β2 promoter. J Biol Chem 273:32200–32212
34. Simons Jr SS, Thompson EB 1981 Dexamethasone 21-mesylate: an affinity label of glucocorticoid receptors from rat hepatoma tissue culture cells. Proc Natl Acad Sci USA 78:3541–3545
35. Lamontagne N, Mercier L, Pons M, Thompson EB, Simons Jr SS 1984 Glucocorticoid *vs.* antiglucocorticoid activity: can a single functional group modification of glucocorticoid steroids always convey antiglucocorticoid activity? Endocrinology 114:2252–2263

36. Ojasoo T, Dore J-C, Gilbert J, Raynaud J-P 1988 Binding of steroid to the progestin and glucocorticoid receptors analyzed by correspondence analysis. J Med Chem 31: 1160–1169
37. Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP 1993 Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol Endocrinol 7:1244–1255
38. Pennie WD, Hager GL, Smith CL 1995 Nucleoprotein structure influences the response of the mouse mammary tumor virus promoter to activation of the cyclic AMP signalling pathway. Mol Cell Biol 15:2125–2134
39. Mercier L, Miller PA, Simons Jr SS 1986 Antiglucocorticoid steroids have increased agonist activity in those hepatoma cell lines that are more sensitive to glucocorticoids. J Steroid Biochem 25:11–20
40. Xu J, Nawaz Z, Tsai SY, Tsai M-J, O'Malley BW 1996 The extreme C terminus of progesterone receptor contains a transcriptional repressor domain that functions through a putative corepressor. Proc Natl Acad Sci USA 93: 12195–12199
41. Xu M, Dittmar KD, Giannoukos G, Pratt WB, Simons Jr SS 1998 Binding of hsp90 to the glucocorticoid receptor requires a specific 7-amino acid sequence at the amino terminus of the hormone-binding domain. J Biol Chem 273:13918–13924
42. Giannoukos G, Silverstein AM, Pratt WB, Simons Jr SS 1999 The seven amino acids (547–553) of rat glucocorticoid receptor required for steroid and hsp90 binding contain a functionally independent lxxll motif that is critical for steroid binding. J Biol Chem 274:36527–36536
43. Onate SA, Tsai SY, Tsai M-J, O'Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270: 1354–1357
44. Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai M-J, Edwards DP, O'Malley BW 1998 The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. J Biol Chem 273:12101–12108
45. Voegel JJ, Heine MJS, Zechel C, Chambon P, Gronemeyer H 1996 T1F2, a 160 kDa transcriptional mediatior for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 15:3667–3675
46. Hong H, Kohl K, Garabedian MJ, Stallcup MR 1997 GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. Mol Cell Biol 17:2735–2744
47. Kazmi SMI, Visconti V, Plante RK, Ishaque A, Lau C 1993 Differential regulation of human progesterone receptor A and B form-mediated trans-activation by phosphorylation. Endocrinology 133:1230–1238
48. Tung L, Mohamed MK, Hoeffler JP, Takimoto GS, Horwitz KB 1993 Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. Mol Endocrinol 7:1256–1265
49. Leonhardt SA, Altmann M, Edwards DP 1998 Agonist and antagonists induce homodimerization and mixed ligand heterodimerization of human progesterone receptors *in vivo* by a mammalian two-hybrid assay. Mol Endocrinol 12:1914–1930
50. Plisov SY, Poirot ME, Modarress KJ, Cavanaugh AH, Edwards DP, Simons Jr SS 1998 Different populations of progesterone receptor-steroid complexes in binding to specific DNA sequences: effects of salts on kinetics and specificity. J Steroid Biochem Mol Biol 67:251–266
51. Petz LN, Nardulli AM 2000 Sp1 binding sites and an estrogen response element half-site are involved in regulation of the human progesterone receptor A promoter. Mol Endocrinol 14:972–985
52. Smith CL, Onate SA, Tsai M-J, O'Malley BW 1996 CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. Proc Natl Acad Sci USA 93:8884–8888
53. McKenna NJ, Nawaz Z, Tsai SY, Tsai M-J, O'Malley BW 1998 Distinct steady-state nuclear receptor coregulator complexes exist *in vivo*. Proc Natl Acad Sci USA 95: 11697–11702
54. Huang Y, Simons Jr SS 1999 Functional analysis of R651 mutations in the putative helix 6 of rat glucocorticoid receptors. Mol Cell Endocrinol 158:117–130
55. Baniahmad A, Dressel U, Renkawitz R 1998 Cell-specific inhibition of retinoic acid receptor-α silencing by the AF2/τc activation domain can be overcome by the corepressor SMRT, but not by NCoR. Mol Endocrinol 12: 504–512
56. Crawford PA, Dorn C, Sadovsky Y, Milbrandt J 1998 Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. Mol Cell Biol 18:2949–2956
57. Zamir I, Zhang J, Lazar MA 1997 Stoichiometric and steric principles governing repression by nuclear hormone receptors. Genes Dev 11:835–846
58. Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson J-A, Carlquist M 1997 Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753–758
59. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95:927–937
60. Simons Jr SS 1987 Selective covalent labeling of cysteines in bovine serum albumin and in HTC cell glucocorticoid receptors by dexamethasone 21-mesylate. J Biol Chem 262:9669–9675
61. Simons Jr SS, Pumphrey JG, Rudikoff S, Eisen HJ 1987 Identification of cysteine-656 as the amino acid of HTC cell glucocorticoid receptors that is covalently labeled by dexamethasone 21-mesylate. J Biol Chem 262: 9676–9680
62. Chrousos GP, Cutler Jr GB, Sauer M, Simons Jr SS, Loriaux DS 1983 Development of glucocorticoid antagonists. J Pharmacol Therap 20:263–281
63. Pons M, Simons Jr SS 1981 Facile, high yield synthesis of spiro C-17-steroidal oxetan-3′-ones. J Org Chem 46: 3262–3264
64. Simons Jr SS, Pons M, Johnson DF 1980 α-Keto mesylate: a reactive thiol-specific functional group. J Org Chem 45:3084–3088
65. Szapary D, Oshima H, Simons Jr SS 1993 A new *cis*-acting element involved in tissue-selective glucocorticoid inducibility of tyrosine aminotransferase gene expression. Mol Endocrinol 7:941–952
66. Simons Jr SS, Miller PA 1984 Comparison of DNA binding properties of activated, covalent and noncovalent glucocorticoid receptor-steroid complexes from HTC cells. Biochemistry 23:6876–6882